

Stochastic modeling of regulation of gene expression by multiple small RNAs

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A wealth of new research has highlighted the critical roles of small RNAs (sRNAs) in diverse processes such as quorum sensing and cellular responses to stress. The pathways controlling these processes often have a central motif comprising of a master regulator protein whose expression is controlled by multiple sRNAs. However, the regulation of stochastic gene expression of a single target gene by multiple sRNAs is currently not well understood. To address this issue, we analyze a stochastic model of regulation of gene expression by multiple sRNAs. For this model, we derive exact analytic results for the regulated protein distribution including compact expressions for its mean and variance. The derived results provide novel insights into the roles of multiple sRNAs in fine-tuning the noise in gene expression. In particular, we show that, in contrast to regulation by a single sRNA, multiple sRNAs provide a mechanism for independently controlling the mean and variance of the regulated protein distribution.

Small non-coding RNAs (sRNAs) are known to play a central role in diverse cellular pathways that bring about global changes in gene expression [1]. In several cases, such global changes are coordinated by a master regulator protein whose expression is controlled by multiple sRNAs [2, 3]. Examples include regulation of the master regulator in bacterial quorum-sensing pathways by multiple sRNAs [4] and regulation of the alternative sigma factor σ^s by four distinct sRNAs, each of which responds to different environmental stresses [2]. Despite its importance in coordinating cellular stress responses and related processes, the role of multiple sRNAs in regulating the expression of a single target gene is not yet well understood [3]. In this work, we address this issue by analyzing a stochastic model that elucidates potential functional roles for this widely-occurring regulatory motif.

Regulation of gene expression by sRNAs is a post-transcriptional process: sRNAs can bind to messenger RNAs (mRNAs) and control protein production by altering mRNA stability or by regulating translational efficiency [1]. The intrinsic stochasticity of the underlying biochemical reactions can produce significant variations ('noise') in gene expression among individual cells in isogenic populations [5–9]. Although noise in gene expression can have deleterious effects in some cases and thus needs to be limited; in other cases such noise is utilized and indeed required by the cell e.g. for processes leading to cell-fate determination [10, 11]. Furthermore, it has been argued that noise in gene expression could be advantageous under conditions of high stress, since variability in a population provides a bet-hedging strategy that can enable survival [12, 13]. Regulation of the noise in gene expression is thus essential for the proper functioning of several cellular processes. Since sRNAs regulate critical cellular processes, understanding their role in fine-tuning the noise in gene expression is of fundamental importance [11].

A quantitative understanding of the cellular functions of sRNAs is aided by the development of models, which can often produce insights that guide future experiments. In recent research, several models for regulation by sRNAs have been developed. Since many sRNAs are known to repress gene expression, most previous models have focused on regulation by irreversible stoichiometric degradation [14–17]. However, sRNAs can affect not only mRNA degradation rates but also protein production rates and the corresponding biochemical reactions are, in general, reversible [1, 18]. Furthermore, not all sRNAs repress gene expression; there are sRNAs which are known to activate gene expression and even some which can switch from activating to repressing in response to cellular signals [18, 19]. To quantify the corresponding effects on stochastic gene expression, a general model which includes the different mechanisms of sRNA-based regulation needs to be analyzed. Such a model, for the case of a single sRNA regulator, has been developed in recent work [20]. Analysis of this model and its extension to multiple sRNAs thus provides a means of addressing outstanding questions about the impact of different modes of regulation by sRNAs on the noise in gene expression.

In this work, we generalize our previous model [20] to analyze the case of multiple sRNAs regulating a single mRNA target. Specifically, we derive exact analytic expressions for the generating function of a protein burst distribution resulting from the regulation of a single target by an arbitrary number of sRNAs. Using this expression, we obtain compact analytic expressions for both the mean and variance of the regulated protein distribution. We first analyze these results for the case of a single regulator and derive insights into different modes of regulation by sRNAs. These results are then contrasted with features unique to regulation by multiple sRNAs. In particular, we show that, in contrast to regulation by a single sRNA, regulation by multiple sRNAs provides the cell

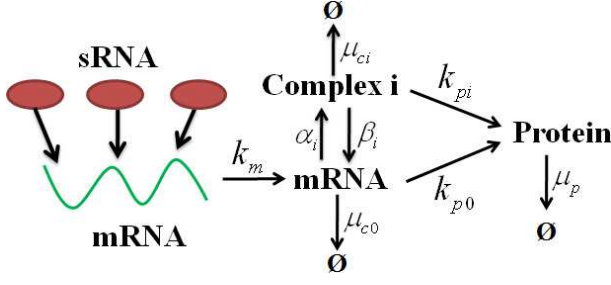


FIG. 1. Schematic illustration of regulation of gene expression by multiple sRNAs. In the full reaction scheme, there are N different regulators and the kinetic scheme is shown for the i^{th} sRNA regulator. The association and dissociation rates for binding to the mRNA are denoted by α_i and β_i respectively. Association results in a complex which produces proteins with rate k_{p_i} and is degraded with rate μ_{c_i} . Note that for the mRNA to transition from one complex to another, it must first return to its unbound state before forming a new complex.

with a mechanism to independently fine-tune both the mean and variance of the regulated protein distribution.

We begin by considering protein production from a single mRNA regulated by N independent sRNAs. The corresponding reaction scheme is shown in Figure 1. The mRNA has $N + 1$ possible states, with the states $i = 1, \dots, N$ denoting mRNA bound to the i^{th} sRNA regulator to form complex i . For notational simplicity, we denote the unbound mRNA state as complex 0. An unbound mRNA forms complex i with rate α_i and the complex can either dissociate with a rate β_i , decay with a rate μ_{c_i} , or initiate protein production with a rate k_{p_i} . We assume the sRNA regulators are present in large amounts such that fluctuations in their concentration can be ignored; correspondingly the rates α_i are taken to be constant.

The distribution of proteins produced from a single mRNA (interacting with N sRNAs) before it decays is denoted as the protein burst distribution $P_{b,N}(n)$. We further define the function $f_i(n, t)$ which denotes the probability that n proteins have been produced and the mRNA is in state i at time t . Correspondingly, the protein burst distribution, $P_{b,N}(n)$, can be obtained from

$$P_{b,N}(n) = \sum_{i=0}^N \int_0^{\infty} f_i(n, t) \mu_{c_i} dt. \quad (1)$$

The time-evolution of the probabilities $f_i(n, t)$ is governed by the Master equation

$$\begin{aligned} \frac{\partial f_0(n, t)}{\partial t} &= k_{p_0}(f_0(n-1, t) - f_0(n, t)) \\ &\quad - (\mu_{c_0} + \sum_{i=1}^N \alpha_i) f_0(n, t) + \sum_{i=1}^N \beta_i f_i(n, t) \\ \frac{\partial f_i(n, t)}{\partial t} &= k_{p_i}(f_i(n-1, t) - f_i(n, t)) \\ &\quad - (\mu_{c_i} + \beta_i) f_i(n, t) + \alpha_i f_0(n, t) \end{aligned} \quad (2)$$

The initial condition corresponds to creation of a single unbound mRNA and no proteins in the system at time $t = 0$, i.e. $f_0(0, 0) = 1$. The procedure outlined in [20] can now be applied to obtain the burst distribution or more precisely the corresponding generating function $G_{b,N}(z) = \sum_n z^n P_{b,N}(n)$. Specifically, defining $F_i(z, s) = \sum_n z^n \int_0^{\infty} e^{-st} f_i(n, t) dt$, we obtain the generating function using

$$G_b(z) = \lim_{s \rightarrow 0} \sum_{i=0}^N \left(\mu_{c_i} F_i(z, s) \right) \quad (3)$$

To present the results in a compact form, it is convenient to define the dimensionless variables $\xi_i = \frac{k_{p_i}}{\beta_i + \mu_{c_i}}$ and $\omega_i = \frac{\alpha_i}{\beta_i + \mu_{c_i}} \left(\frac{\mu_{c_i}}{\mu_{c_0}} \right)$ for $i > 0$ and $n_i = \frac{k_{p_i}}{\mu_{c_i}}$ for $i \geq 0$. Now, by setting $\omega_0 = 1$ and $\xi_0 = 0$ we further define the ‘weight functions’ $\omega_i(z) = \omega_i \frac{1}{1 + \xi_i(1-z)}$. Note that $\frac{1}{1 + \xi_i(1-z)}$ is the generating function of a geometric distribution with mean ξ_i .

Using the above definitions, we obtain the following exact expression for the generating function of the protein burst distribution

$$G_{b,N}(z) = \frac{\sum_{i=0}^N \omega_i(z)}{\sum_{i=0}^N \omega_i(z) + \sum_{i=0}^N \omega_i(z) n_i (1-z)} \quad (4)$$

For $N = 0$, i.e. the unregulated case, the generating function reduces to

$$G_{b,0}(z) = \frac{1}{1 + n_0(1-z)} \quad (5)$$

in agreement with previous work showing that the protein burst distribution is a geometric distribution with mean $n_0 = \frac{k_{p_0}}{\mu_{c_0}}$ [20, 21]. Eq. (4) provides the generalization of this result for the case of N sRNA regulators using the weight functions $\omega_i(z)$.

An important mechanism of regulation by sRNAs corresponds to the case that sRNA binding prevents ribosome access and thus blocks translation. For the case that all the regulators act to fully repress translation, i.e. $k_{p_i} = 0$ for $i > 0$, we have $\omega_i(z) = \omega_i$. Correspondingly, the generating function reduces to the formula for the unregulated case (Eq. (5)) with a renormalized mean given by $\frac{n_0}{\sum_{i=0}^N \omega_i}$. This interesting observation indicates that regulation by sRNAs which function by fully repressing translation is reversible: for arbitrary concentrations of the sRNA regulator, by appropriately adjusting the parameter k_{p_0} (e.g. by adjusting the concentration of ribosomes), the regulated protein distribution in the presence of sRNAs can be made identical to the distribution for the unregulated case (prior to introduction of the sRNAs).

For the general case, the generating function can be recast in a form that shows that the protein burst distribution is a mixture of $N + 1$ geometric distributions [20].

However, the corresponding expression, even for the case of $N = 2$, is too complex to be reproduced here. On the other hand, using Eq. (4), compact analytic expressions for the mean, n_{b_N} , and squared coefficient of variance, $\sigma_{b_N}^2/n_{b_N}^2$ can be derived. The mean (scaled by the unregulated mean) is given by

$$\frac{n_{b_N}}{n_{b_0}} = 1 + F_N \quad (6)$$

and the noise strength (squared coefficient of variance) is given by

$$\frac{\sigma_{b_N}^2}{n_{b_N}^2} = 1 + \frac{1}{n_{b_N}} + Q_N \quad (7)$$

where

$$F_N = \frac{\sum_{i=0}^N \omega_i (n_i - n_0)}{\sum_{i=0}^N \omega_i n_0}$$

$$Q_N = \frac{\sum_{i,j=0}^N \omega_i \omega_j (\xi_i - \xi_j)(n_i - n_j)}{\left(\sum_{i=0}^N \omega_i n_i\right)^2}$$

Note that the signs of F_N and Q_N characterize the impact of the sRNAs on the regulated protein distribution. Specifically, the unregulated case has mean n_{b_0} ; thus $F_N < 0$ corresponds to repression whereas $F_N > 0$ corresponds to activation. Similarly, an unregulated protein burst distribution with mean n_{b_N} has a squared coefficient of variance $1 + 1/n_{b_N}$; thus, when $Q_N < 0$ we have noise reduction whereas $Q_N > 0$ corresponds to increased noise strength (relative to an unregulated burst distribution with the same mean).

We now focus on using Eq. (6) and Eq. (7), to elucidate interesting features for the case of regulation by a single sRNA, i.e. $N = 1$. Note that all of the variables in the expressions for the mean and noise strength are always positive (or zero) except for the term $(n_1 - n_0)$. Thus, the sign of F_1 and Q_1 is determined completely by $\Delta_{10} = n_1 - n_0$. When $\Delta_{10} > 0$ both the mean and the noise strength are higher than their unregulated values. Similarly, when $\Delta_{10} < 0$ both the mean and the noise strength are lower than the corresponding unregulated values (except for the case $\xi_i = 0$ for which the noise strength is identical to an unregulated distribution with the same mean). In either case, we note that, for a single sRNA regulator, there is a coupling between the mean and noise strength of the regulated burst distribution such that both cannot be tuned independently, e.g. a decrease in the mean cannot be associated with an increase in the noise strength.

In contrast to the case of regulation by a single sRNA, in the case of regulation by multiple sRNAs, the mean and noise of the protein distribution can be tuned independently. The deviation of the mean from its unregulated value depends solely upon terms of the form

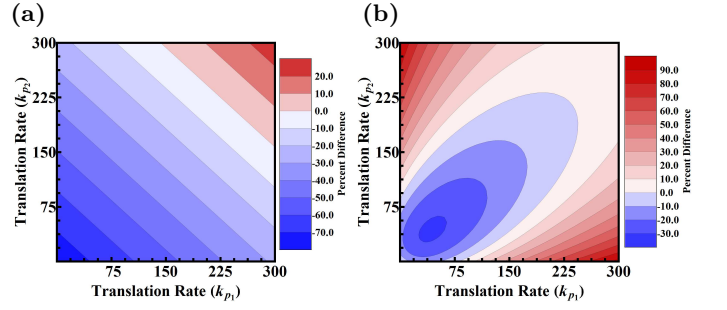


FIG. 2. Contour plots for the percent change in the mean and noise strength of a two regulator system from the corresponding unregulated values as a function of k_{p1} and k_{p2} . (a) Mean: Plot of $f(k_{p1}, k_{p2}) = \frac{n_{b2} - n_{b0}}{n_{b0}} \cdot 100\%$. Note that along the contour $f(k_{p1}, k_{p2}) = -20\%$ the noise strength changes from less than -5% to over 70% . (b) Noise Strength: Plot of $g(k_{p1}, k_{p2}) = \frac{Q_2}{1 + 1/n_{b2}} \cdot 100\%$. Note that $g(k_{p1}, k_{p2})$ contains contours that sweep out a large portion of the plotted (k_{p1}, k_{p2}) state space. By proportionally changing the k_p values corresponding to the two regulators, the noise strength can be varied while maintaining the same mean value. The parameters used were $k_{p0} = 50$, $\mu_{c0} = 1$, $\mu_{c1} = 4.5$, $\mu_{c2} = 4.5$, $\beta_1 = 1$, $\beta_2 = 0.5$, $\alpha_1 = 2$ and $\alpha_2 = 2$.

$\Delta_{i0} = n_i - n_0$. On the other hand, considering the general form of the noise strength for $N > 1$, we have terms of the form $\tilde{\Delta}_{ij} = (\xi_i - \xi_j)(n_i - n_j)$ that contribute to the deviation from the corresponding unregulated value. Thus, for appropriately chosen parameters, two sRNAs can be used to tune both the mean and variance of the regulated protein distribution as discussed below.

Consider the case of regulation by 2 sRNAs that are maintained at some fixed cellular concentrations. A new mRNA target for these sRNAs can arise from the evolution of appropriate sRNA binding sites on the mRNA sequence. For the new target, we assume that the parameters k_{p1} and k_{p2} can be tuned based on changes in the sequence and location of the sRNA binding sites. The corresponding variation in the mean and noise strength is shown in Fig. 2. Note that by maintaining a linear relationship between k_{p1} and k_{p2} , the mean of the regulated protein distribution can be left unchanged; however, the noise strength can be tuned over a large range. For example, for some choices of the parameters, the mean can be fixed and the noise strength can be varied by over 100% relative to the unregulated distribution (see Fig. 2). In this context, it is interesting to note that it has been observed that several sRNAs have a minimal effect on the mean levels of their regulatory targets. For such targets, sRNAs could be functioning primarily as modulators of noise while giving rise to only a minimal change in mean levels due to regulation [11]. Our results provide quantitative insight into how such regulation can be implemented using multiple sRNA regulators.

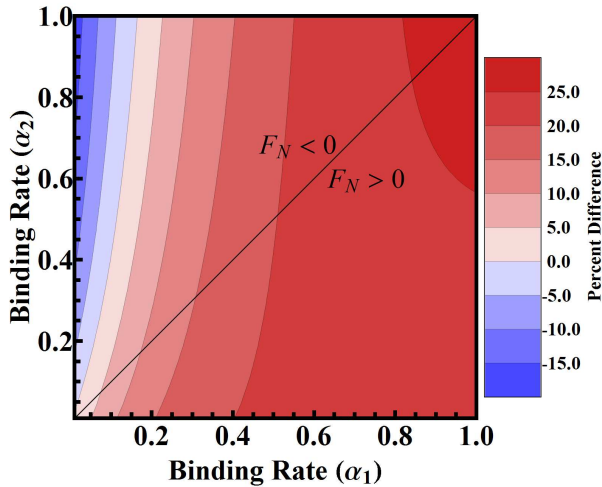


FIG. 3. Contour plot of the percent change in noise strength of a two regulator pathway from its corresponding unregulated value as a function of α_1 and α_2 , i.e. $f(\alpha_1, \alpha_2) = \frac{Q_2}{1+1/n_{b2}} \cdot 100\%$. The change in mean from the unregulated to regulated pathways, F_N , is positive below and negative above the line $\alpha_1 = \alpha_2$. The parameters used were $k_{p0} = 50$, $k_{p1} = 200$, $k_{p2} = 72.5$, $\mu_{c0} = 1$, $\mu_{c1} = 2.725$, $\mu_{c2} = 2.725$, $\beta_1 = 0.15$ and $\beta_2 = 0.15$.

The results obtained also illustrate how changing sRNA concentrations can be used to modulate the noise in gene expression. For our model, changes in the concentration of the sRNA regulators effectively alter the binding rates (α_i) to the mRNA. From Eq. (6), we see that for 2 regulators, by choosing one of the regulators to be a repressor and the other to be an activator, the mean of the regulated protein distribution can be increased ($F_N > 0$) or decreased ($F_N < 0$) by adjusting the relative concentrations of the two regulators. Furthermore, by changing the concentrations of the regulators such that their relative concentration is fixed, the mean of the regulated protein distribution is left unchanged, whereas the variance can be tuned over a range of values. This insight is particularly relevant, given that noise can be advantageous to a cell, especially in response to stress.

Finally, we note that while the above analysis focuses on the burst distribution from a single mRNA, the results are readily generalized to the case of arbitrary mRNA burst distributions, assuming independent contributions from each mRNA [22]. Assuming independent bursts and using equations derived in recent work [22], we can use the derived results to obtain corresponding expressions for the mean and variance of the steady-state protein distribution. A detailed analysis of the connection to steady-state distributions will be presented elsewhere.

Modulation of gene expression in pathways containing multiple sRNA regulators could provide a multitude of selective advantages in specific contexts and our model provides a means of gaining insight into these processes. In particular, our analysis shows how by adjusting the

concentrations of multiple sRNA regulators, a cell can initiate finely tuned responses to external stimuli. This could explain the ubiquity of sRNA regulators in cellular stress response, a process for which gene expression noise is known to be critical. In a broader context, the fine control afforded to cells via regulation by multiple sRNAs could be useful in signal integration when multiple environmental stimuli are present simultaneously. This work provides a basis for future work investigating such signal integration and response in more complex pathways; thus opening new avenues for understanding and modeling stochastic gene expression in a wider class of regulatory networks.

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